HPV prevalence and type-distribution in cervical cancer and premalignant lesions of the cervix: A population-based study from Northern Ireland

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ABSTRACT:

Introduction: Assessment of Human papillomavirus (HPV) prevalence and genotype distribution is important for monitoring the impact of prophylactic HPV vaccination. This study aimed to demonstrate the HPV genotypes predominating in pre-malignant and cervical cancers in Northern Ireland (NI) before the vaccination campaign has effect.

Methods: Formalin fixed paraffin embedded tissue blocks from 2,303 women aged 16-93 years throughout NI were collated between April 2011 and February 2013. HPV DNA was amplified by PCR and HPV genotyping undertaken using the Roche® linear array detection kit.

Results: In total, 1,241 out of 1,830 eligible samples (68.0%) tested positive for HPV, with the majority of these [1,181/1,830 (64.5%)] having high-risk (HR) HPV infection; 37.4% were positive for HPV-16 (n=684) and 5.1% for HPV-18 (n=93). HPV type-specific prevalence was 48.1%, 65.9%, 81.3%, 92.2% and 64.3% among cervical intraepithelial neoplasias (CIN) grades I-III, squamous cell carcinomas (SCC) and adenocarcinoma (AC) cases, respectively. Most SCC cases (81.3%) had only one HPV genotype detected and almost a third (32.0%) of all cervical pathologies were HPV negative including 51.9% of CIN I (n=283), 34.1% CIN II (n=145), 18.7% of CIN III (n=146), 7.8% of SCC (n=5) and 35.7% of AC (n=5) cases.

Conclusions: This study provides important baseline data for monitoring the effect of HPV vaccination in NI and for comparison with other UK regions. The coverage of other HR-HPV genotypes apart from 16 and 18, including HPV-45, 31, 39 and 52, and the potential for cross protection, should be considered when considering future polyvalent vaccines.

Keywords: human papillomavirus, cervical cancer, cervical intraepithelial neoplasia, frequency, population-based.

INTRODUCTION

Annually 3,000 women are diagnosed with, and almost 1,000 die from cervical cancer in the United Kingdom (UK), making it the 12th most common cancer diagnosis and 17th most common cancer death [Cancer Research UK, 2014]. Two main subtypes exist, squamous cell carcinoma (SCC) and adenocarcinoma (AC). Persistent infection with high-risk (HR) oncogenic human papillomavirus (HPV) family *alpha-Papillomaviridae* is a necessary, but insufficient cause, for invasive cervical cancer [IARC Working Group on the Evaluation of Carcinogenic Risks to Humans., 2012]; with HPV-16 and HPV-18, the most common oncogenic types, contributing to around 70% of all cases worldwide [WHO/ICO Information Centre on HPV and Cervical Cancer, 2010].

HPV is highly sexually transmissible with 80% of women expected to have a HPV infection at some point during their sexually active life [Alexander *et al*, 2012]. Many women are repeatedly infected, with most infections occurring in those aged under 25 years [de Sanjosé *et al*, 2007; Smith *et al*, 2008; Anderson *et al*, 2013]. However, despite frequent exposure to HPV, development of cervical cancer is uncommon with most HPV infections being transient and spontaneously regressing within two years with no residual abnormality [Castellsagué, 2008]. In some cases HPV infection may persist [Trottier *et al*, 2009], with some HPV genotypes more persistent than others [Louvanto *et al*, 2010]. Recent studies indicate a higher proportion of CIN II and CIN III lesions progressing to invasive cancer in HPV-16 positive lesions, often within as little as 10-20 years [Jaisamrarn *et al*, 2013; Vink *et al*, 2013; Wentzensen *et al*, 2013]. Many aspects of the natural history of HPV infection remain to be fully elucidated, including the probable existence of latent infections and potential reactivation.

Although HPV-16, 18, 31, 52 and 58 are cited among the top 10 most common HPV genotypes worldwide [Bruni *et al*, 2010], there is significant variation in the reported prevalence of HR-HPV genotypes between countries. We previously reported that the prevalence of any HPV infection in Northern Ireland (NI) women having a pap smear was 17.1%, ranging from 42.5% in those aged 20-

24 years to 4.2% in women aged 60-64 years; HPV prevalence was lower in women with normal cytology at 13.2% increasing in those with cervical disease [Anderson *et al*, 2013]. However, this study was underpowered to detect the prevalence of HPV genotypes in high-grade cervical abnormalities (n=72).

The aim of the present study was therefore to provide an estimate of the background prevalence of HPV infection and age-specific HPV type distribution, and to highlight the HPV genotypes most frequently present in cervical cancer tissue and precursor lesions, before the HPV vaccination programme alters the distribution of HPV infection in NI. Of note, unpublished data from the present study has been recently incorporated into a pooled analysis of the HPV type-specific prevalence in invasive cervical cancers in the UK [Mesher *et al*, 2014] where the overall prevalence of any HR-HPV was found to be 95.8%; data that will be valuable in future HPV vaccine monitoring and effectiveness studies. More detailed data on the prevalence of all detected HPV genotypes stratified by five year age-bands, inclusive of CIN I-III and cervical pathologies are reported herein.

METHODS

Sample selection

Prospectively derived formalin fixed paraffin embedded (FFPE) cervical tissue blocks (processed at all four pathology laboratories in NI) pertaining to routine diagnostic samples collected between April 2011 and February 2013 from all women attending for cervical screening across NI (population-based cohort), and which were determined to have cervical abnormalities, were eligible for inclusion. No age or histological restrictions were applied to the cohort during specimen collection.

Through data supplied by the Public Health Agency Northern Ireland, we estimate that only a small proportion of samples may have been derived from previously HPV vaccinated women (5.9%), thus the majority of samples included in this study are from HPV unvaccinated women. Following routine examination of the biopsy in each reporting laboratory, consultant pathologists selected the most appropriate FFPE block containing the highest grade of disease reported. The selected cervical tissue blocks were sent to the Department of Cellular & Molecular Pathology, Northern Health & Social Care Trust for HPV determination. For the purposes of the present study, analysis was restricted to samples identified as cervical intraepithelial neoplasia (CIN) grades I, II and III, SCC and AC. On reception of the FFPE tissue blocks, pathology records were accessed and clinical pathological information including patient age, sample type and histology, were extracted. A unique study number was then assigned to each case and the link to the original case broken, ensuring confidentiality and complete anonymisation of the results. Ethical approval for the study was granted from the Office of Research Ethics Committees NI (ORECNI ref: 08/NIIR02/104).

Specimen processing

FFPE tissue blocks underwent tissue microtomy and were processed under molecular pathology conditions, to reduce the risk of cross contamination. Tissue lysis was performed using the QIAGEN ATL (EDTA and 10% sodium dodecyl sulphate) buffer (P/N 19076, Qiagen, Crawly, West Sussex, UK),

in the presence of 20µl of proteinase K (P/N 19131, Qiagen, Crawley, West Sussex, UK). The tissue was incubated at 56°C ± 2°C for 24 hours with occasional vortex mixing during this incubation period. During post enzymatic incubation, the lysate was added to processing tubes and was subjected to automated DNA extraction on the Roche COBAS® 480 X module as per manufactures protocols. Extracted DNA was eluted into buffer EB and stored at -20°C until amplification. Detection of HPV genotypes were undertaken using a modified Roche Linear Array HPV genotyping test (LA HPV GT P/N:04391853 190) and the Linear Array detection kit (LA DK P/N: 03378179 190, Roche Molecular Systems, Inc. Branchburg, NJ, USA). The modification from manufactures protocol consisted of the addition of a compound into the HPV MasterMix, to allow for compatibility with the DNA extracted from the COBAS® 480 X module. Extracted DNA was quantified via NanoDrop spectrophotometry with all samples standardised to a concentration of 24ng/ul; the optimum level determined in-house to allow for successful HPV detection in the background of human DNA and reduce the number of test failures due to an "overloaded" PCR reaction. Of note, the Roche linear array uses multiple type (mixed) probes to detect DNA from HPV-52 which limits the assay's ability to discriminate HPV-52 status in the presence of HPV 33, 35 and/or 58 infections. The HPV-52 genotype was thus derived as positive if co-infection of HPV 33, 35 and 58 was not present in the sample. Cervical SCC samples that tested HPV negative were revisited, DNA isolated and retested to ensure the result. Samples were considered HPV negative only when a linear array negative result was obtained and confirmed by repeat testing.

Specimen Reporting

The term HR-HPV is used to describe a number of HPV genotypes that have been shown to be associated with an increased risk of cervical cancer and include: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 (carcinogenic), 68 (probably carcinogenic) and 66 (possibly carcinogenic) based on the World Health Organization (WHO) HPV categorizations [Cogliano *et al*, 2005]. The term 'low-risk

HPV' covers all other HPV genotypes detected by the Roche HPV linear array genotyping test. All HPV types identified by the Roche linear array are shown in Table 3.

Statistical analysis

The overall prevalence, genotype and age-specific HPV prevalence among pre-malignant lesions (CIN I-III) and cervical cancers (SSC and AC) were estimated for all samples. The frequency of those testing positive for HPV-16 and HPV-18 with or without other HR-HPV genotypes was investigated. Women within the study were stratified into five year age bands, this was further classified into three age groups for the following reasons; under 25 years (outside the age range for the current NI Cervical Screening Programme, but current at the time of our previous prevalence study in the screened population); 25 to 34 years (reported to have a higher transient HPV infection risk with no significant underlying disease [Khan *et al*, 2005]), over 35 years (this group of HR-HPV positive is said to have a higher chance of significant pathological outcome [Khan *et al*, 2005]). STATA IC/11.2 (StataCorp, College Station, TX, USA) was used for all analyses.

RESULTS

In total, 2,303 samples were received between April 2011 and February 2013. Figure 1 is a flow diagram providing justification for the selection of study samples. Exclusions included 58 samples that were not tested due to overrepresentation in the low-grade dyskaryosis groups, a further 200 were excluded at the post-analytical phase of the study due to insufficient tissue remaining for molecular analysis. 218 samples were also excluded as they were not SCC, AC or CIN I-III pathologies. A total of 1,827 eligible samples were included for analysis, the majority of which were acquired through large loop excisions of the transformation zone (64.8%) or punch biopsies (34.2%).

Prevalence of HPV infection:

In total, 1,243/1,827 samples (68.0%) tested positive for HPV, with the majority 1,183/1,827 (64.8%) having HR-HPV infection. A total of 584/1,827 (32.0%) of samples were HPV negative, 37.4% were positive for HPV-16 (n=684) and 5.1% for HPV-18 DNA (n=93). Figure 2 outlines the overall HPV genotype profile among the cohort and HPV multiplicity. The five most common HPV genotypes detected across all cervical pathologies examined were HPV-16 (n=684), HPV-31 (n=150), HPV-52 (n=125), HPV-18 (n=93) and HPV-33 (n=91) (Figure 2).

HPV type-specific prevalence:

The majority of samples were from CIN III cases (42.6%). HPV DNA was detected in 262/545 (48.1%) of CINI samples, 280/425 (65.9%) of CIN II lesions, 633/779 (81.3%) of CIN III specimens, 59/64 (92.2%) of SCCs and 9/14 (64.3%) of AC samples. Table 1 details the number of HPV genotypes detected by pathological subtype. Half of all samples (49.7%) had only one HPV genotype detected (n=908). Multiple HPV genotypes (>1) were more common in CIN I-III lesions than other cervical pathologies. Among SCC specimens, 7.8% had no HPV DNA detected and most (81.3%) had only 1 HPV genotype persisting, Table 1. HPV-16 and/or HPV-18 DNA was present in the majority of SCC (82.8%) and AC (64.3%) cases and over half (56.2%) of CIN III samples, Table 2. Other HR HPV

genotypes were more prevalent in CIN I-III pathologies, and present in less than 10% of SCCs and AC cases. LR-HPV genotypes were most common in lower grade cervical lesions and absent in cervical cancer specimens, Table 2. Almost a third (32.0%) of all pathologies were HPV negative. The distribution of HPV genotypes detected within each cervical pathology is detailed in Table 3. The current HPV vaccination genotypes (HR-HPV 16 and or HR-HPV 18) were found in at least 82.8% of women with SCC of the cervix. For cervical SCC, HPV-16 (n=51/64), HPV-18 (n=3/64), HPV-45 (n=3/64) and HPV-52 (n=5/64) made up 96% of the HPV positive samples. HPV-16 was the most common HPV detected across all cervical pathologies, except for AC where HPV-18 predominated with HPV-16, HPV-51 and HPV-54 making up the remaining HPV genotypes present, Table 3.

Age-specific prevalence for HPV infection:

The pathological distribution of samples by five-year age group is shown in Table 4. The mean age of women included in the study was 32 years (range 16-93 years, standard deviation (SD) 9.4 years) and significantly differed between histopathological groups (P <0.001). In total, 375 samples were from women aged 24 years and under, 887 from women aged 25 to 34 years and 565 samples from those aged 35 years and over. CIN I was more common in younger women and CIN II and CIN III most common in those aged 25-29 years. SCC was most common in women aged 35-39 years, Table 4.

The number of HPV genotypes decreased with increasing age, with just over half (51.0%) of all HPV infections found in tissue from women aged between 25-29 years, Table 1. Most tissue samples had a single HPV genotype and this was more common in those aged over 60 years. Five or more HPV genotypes were detected in some women but this was largely limited to those aged under the age of 40 years, Table 1. 41.7% of women with cervical pathology had HPV-16 or HPV-18 detected, and the highest proportion HPV 16/18 positive were over the age of 65 years, Table 2. Other HR-HPV genotypes were more common in younger women, particularly those aged 35-39 years. LR-HPV

genotypes were most common in those aged 55-59 years, with women over the age of 60 years having no LR-HPV genotypes detected, Table 2.

DISCUSSION:

This is the largest population-based study investigating the HPV genotype distribution in women with CIN I-III and cervical SCC and AC in NI. The overall prevalence of any HPV in SCC samples was 92.2% and was 68.0% across all cervical samples investigated. HR-HPV was detected in 64.8% of all samples with HPV-16 being the most common HPV genotype identified, which is consistent with that described elsewhere in CIN cases across Europe in countries such as Spain, France and Germany [García-Espinosa *et al*, 2012; Monsonego *et al*, 2012; de Jonge *et al*, 2013; Leinonen *et al*, 2013; Rössler *et al*, 2013] and in invasive disease internationally [Bosch *et al*, 2008; de Sanjose *et al*, 2010; Li *et al*, 2011]. As previously reported the prevalence of HPV increased through CIN I-III lesions in NI [Anderson *et al*, 2013] from 48.1% in CIN I to 81.3% in CIN III cases. Most HPV positive samples had HR-HPV genotypes with HPV-16 and/or HPV-18 present in 20.6%, 35.3% and 56.2% of CIN I-III cases respectively.

In an examination of FFPE tissue from more than 6,000 women from 17 European countries using the SPF10-LiPA25 assay, including data from neighbouring Scotland, Ireland and Wales, Tjalma *et al* found HPV-16 was the most frequent HPV type detected in both CIN and invasive cervical cancer [Tjalma *et al*, 2013]. HPV-16 and/ or HPV-18 prevalence (among HPV positive cases) was reported as 45.8% in CIN II and 67.3% in CIN III cases [Tjalma *et al*, 2013], slightly higher than the current study. The authors reported HPV-31, 33, 35, 51, 52, 58 and 68 as the most frequently detected genotypes in women with high-grade CIN lesions. Apart from HPV-16, we similarly found that HPV-18, 31, 33, 45, 51 and 52 were the most common genotypes identified in high grade lesions. Building on previous meta-analyses of type-specific HPV prevalence worldwide by specific grades of cervical disease [Clifford *et al*, 2003, 2005; Smith *et al*, 2007; Li *et al*, 2011] Guan *et al* in a further meta-analysis of 423 studies (144 of which were from Europe) using PCR assays based on various primers among cell or biopsy/tissue cervical diagnoses also showed an increasing HPV prevalence with increasing severity of cervical disease, with HPV-16 the most frequently detected HR-HPV type in all

grades [Guan *et al*, 2012]. The prevalence (including HPV negative cases in the denominator) of HPV-16 was 27.6%, 39.8% and 58.2% and HPV-18 prevalence 9.0%, 10.0% and 7.4% in CIN I to CIN III cases respectively [Guan *et al*, 2012], again higher than the estimates reported in the present study, variation which may have arisen from methodological differences in HPV detection techniques. The majority of SCCs (92.2%) in the present study were HPV positive, this is in accordance with the proportions of invasive cervical cancers (the majority of which were SCCs) testing HPV positive in other countries of the UK [Mesher *et al*, 2014], Sweden, Spain Italy and eastern Europe [Du *et al*, 2011; Alemany *et al*, 2012; Giorgi Rossi *et al*, 2012; Guan *et al*, 2012; Piana *et al*, 2013; Pista *et al*, 2013; Škamperle *et al*, 2013; Tjalma *et al*, 2013; Kjær *et al*, 2014; Simanaviciene *et al*, 2014].

There were only 14 cases of AC included in the current investigation, and therefore our study is likely underpowered to investigate HPV prevalence in this subgroup. Of note however, HPV-18 was the most common HPV type in AC cases with a prevalence of 42.9% in the current study, akin to a recent Scottish estimate of 44.0% (among HPV positive cases) [Powell *et al*, 2013] which examined HPV DNA in FFPE tissue detected using the SPF10-DEIA/LiPA25-PCR assay. The prevalence of HPV-16 and/or HPV-18 was 64.3%, lower than reported in an English multi-site investigation of HPV DNA in cervical cytology and cervical cancer biopsies using the Roche Linear array typing system (81.9%, among HPV positive cases) [Howell-Jones *et al*, 2010] and among AC cases from other European studies (94.6%) [Tjalma *et al*, 2013]; perhaps due to a lower HPV-18 rate.

The number of HPV genotypes detected in the current study varied across pathological grade, with the lowest percentage of single genotypes (31.0%) in CIN I lesions and the highest proportion (81.3%) in cervical SCCs, indicative that a single HPV genotype remains persistent in patients that progress. Although HPV persistence has been shown to vary by geographical region, HPV-16, 18, 31, 33, 45, 52 and 58 are the most persistent HPV genotypes reported among women with invasive cervical cancer [Bernard *et al*, 2013; Rositch *et al*, 2013]. The most frequent single genotypes in the

current investigation in SCCs were HPV 16, 18, 45, 31, 39 and HPV 52. Notably, this profile includes several HPV genotypes which are not currently incorporated in the present HPV vaccine programme. These should be considered in the next generation of anti-HPV vaccines. In line with the UK HPV vaccination programme, in September 2012 NI began using the quadrivalent vaccine Gardasil® (Gardasil, Sanofi Pasteur MSD), with a relatively high uptake of 86.8% for all three doses in girls aged 12-13 years [HSC Public Health Agency Northern Ireland, 2014]. Markov modelling has shown that the UK HPV vaccination programme would require 80% uptake to have a reduction of 66% in the prevalence of high-grade precancerous lesions and a 76% reduction in cervical cancer deaths [Kohli *et al*, 2007]. Therefore the HPV genotypes covered by the current HPV vaccination programme will lower the prevalence of HPV 16/18, as has already been evidenced in Scotland [Kavanagh *et al*, 2014], and should prevent the majority of cervical cancers in NI. The formerly used bivalent vaccine (Cervarix®) offers some cross-protection for HPV genotypes of the A7 species including HR HPV-31, 33 and 45 [Paavonen *et al*, 2009; Malagón *et al*, 2012; De Vincenzo *et al*, 2013; Verdenius *et al*, 2013]. The potential effects of vaccine cross protection against other oncogenic non-target genotypes should also be considered when conducting future cost-benefit analyses.

The principal strength of this study is its size and population-based design. The study was able to report the identification of high and low-risk HPV genotypes as well as the prevalence of multiple HPV infections. When comparing the HPV prevalence between countries it is important to consider that variations in HPV positivity may be explained by differences in the quality and type of samples analyzed (biopsies, surgical specimens or fresh tissue), as well as the methods of HPV detection and assessment.

While it is frequently reported that 99.7% of cervical SCCs are HPV positive [Walboomers *et al*, 1999], women have been shown to have cervical disease without testing HPV positive using current methods. In a pooled analysis of 3 large Italian case series using three different PCR methods, 24

(4.2%) of 574 invasive cervical cancers were found to be 'true' HPV negative cases [Giorgi Rossi *et al*, 2012]. Similarly, Tjalma *et al* in large-scale data from 17 European countries found that 8.2% of invasive cervical cancers were HPV negative [Tjalma *et al*, 2013]. HPV-negative carcinoma, if it exists at all, is likely to be rare. In the present study 10/78 invasive cervical cancers (12.8%) tested HPV negative. Although other aetiologic factors, such as mutations within the p53 gene, may explain some HPV negative cases [Fogel *et al*, 1995] the potential for false negative results arising from differences in analytic sensitivity for different HPV types particularly in the presence of multiple infections, low titer or copy number of HPV DNA and inadequacy of the specimen should be carefully considered. For instance, HPV genotyping analysis in the present study was undertaken using the Roche linear array detection kit. Importantly, this assay uses PGMY L1 consensus primers to amplify a 450-bp fragment. As it is well known that formaldehyde fixation provides low yields of extractable DNA due to protein cross-linking and strand cleavage, such a large amplicon size provides the potential for false negative results on FFPE tissue. Whilst the detection of HPV in fresh frozen tissue appears superior [Odida *et al*, 2010], this would have been difficult to acquire and process in a large population-based study such as this.

In conclusion, HPV-16 was identified as the main HPV genotype associated with cervical disease in NI, contributing to around 83.0% of the cervical SCCs investigated. Provided there is sustained high HPV vaccine coverage in NI, the current HPV vaccination programme should prevent the majority of cervical cancers but coverage of other HR-HPV genotypes with high prevalence and oncogenic potential including HPV-31, 39, 45 and 52 and the potential influence of cross protection, should be considered in any future polyvalent vaccines.

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FIGURE LEGENDS:

Figure 1: Flowchart of sample selection and eligibility

Figure 2: Overall HPV genotype profile denoting the prevalence of single and multiple HPV infections

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Table 1: Percentage distribution of the number of HPV genotypes detected by pathological subtype and 5-year age group.

Number of HPV Genotypes detected (% of each pathology)							
Pathology	**HPV negative**	**1**	**2**	**3**	**4**	**5 or more**	**Total** n (%)
CIN I	283 (51.9)	169 (31.0)	63 (11.6)	19 (3.5)	9 (1.7)	2 (0.4)	545 (100.0)
CIN II	145 (34.1)	217 (51.1)	46 (10.8)	15 (3.5)	1 (0.2)	1 (0.2)	425 (100.0)
CIN III	146 (18.7)	462 (59.3)	129 (16.6)	32 (4.1)	4 (0.5)	6 (0.8)	779 (100.0)
SCC	5 (7.8)	52 (81.3)	6 (9.4)	1 (1.6)	0 (0.0)	0 (0.0)	64 (100.0)
AC	5 (35.7)	8 (57.1)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	14 (100.0)
Age group (yrs)	**HPV negative**	**1**	**2**	**3**	**4**	**5 or more**	**Total** n (%)
Under 25	115 (30.7)	172 (45.9)	64 (17.1)	18 (4.8)	3 (0.8)	3 (0.8)	375 (100.0)
25-29	152 (28.7)	270 (51.0)	79 (14.9)	21 (4.0)	6 (1.1)	1 (0.2)	529 (100.0)
30-34	110 (30.7)	186 (52.0)	44 (12.3)	14 (3.9)	1 (0.3)	3 (0.8)	358 (100.0)
35-39	74 (33.3)	119 (53.6)	23 (10.4)	4 (1.8)	1 (0.5)	1 (0.5)	222 (100.0)
40-44	51 (33.8)	77 (51.0)	16 (10.6)	6 (4.0)	1 (0.7)	0 (0.0)	151 (100.0)
45-49	41 (44.6)	39 (42.4)	9 (9.8)	2 (2.2)	1 (1.1)	0 (0.0)	92 (100.0)
50-54	24 (49.0)	17 (34.7)	6 (12.2)	1 (2.0)	1 (2.0)	0 (0.0)	49 (100.0)
55-59	10 (35.7)	13 (46.4)	3 (10.7)	1 (3.6)	0 (0.0)	1 (3.6)	28 (100.0)
60-64	3 (33.3)	6 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (100.0)
65+	4 (28.6)	9 (64.3)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	14 (100.0)
TOTAL	**584 (32.0)**	**908 (49.7)**	**244 (13.4)**	**68 (3.7)**	**14 (0.8)**	**9 (0.5)**	**1,827 (100.0)**

CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous cell carcinoma, AC = Adenocarcinoma

Table 2: Type of HPV genotypes detected by cervical pathology and 5-year age group.

Pathology	HPV-16 and or HPV-18 n (%)	Other High-risk HPV genotypes* n (%)	Low risk HPV genotypes Only n (%)	HPV negative n (%)	Total n (%)
CIN I	112 (20.6)	121 (22.2)	29 (5.3)	283 (51.9)	545 (100.0)
CIN II	150 (35.3)	110 (25.9)	20 (4.7)	145 (34.1)	425 (100.0)
CIN III	438 (56.2)	184 (23.6)	11 (1.4)	146 (18.7)	779 (100.0)
SCC	53 (82.8)	6 (9.4)	0 (0.0)	5 (7.8)	64 (100.0)
AC	9 (64.3)	1 (7.1)	0 (0.0)	5 (35.7)	14 (100.0)
Age group (yrs)					
Under 25	154 (41.1)	93 (24.8)	13 (3.5)	115 (30.7)	375 (100.0)
25-29	237 (44.8)	127 (24.0)	13 (2.5)	152 (28.7)	529 (100.0)
30-34	151 (42.2)	82 (22.9)	15 (4.2)	110 (30.7)	358 (100.0)
35-39	84 (37.8)	59 (26.6)	5 (2.3)	74 (33.3)	222 (100.0)
40-44	59 (39.1)	36 (23.8)	5 (3.3)	51 (33.8)	151 (100.0)
45-49	34 (37.0)	12 (13.0)	5 (5.4)	41 (44.6)	92 (100.0)
50-54	18 (36.7)	5 (10.2)	2 (4.1)	24 (49.0)	49 (100.0)
55-59	12 (42.9)	4 (14.3)	2 (7.1)	10 (35.7)	28 (100.0)
60-64	4 (44.4)	2 (22.2)	0 (0.0)	3 (33.3)	9 (100.0)
65+	9 (64.3)	1 (7.1)	0 (0.0)	4 (28.6)	14 (100.0)
TOTAL n (%)	**762 (41.7)**	**421 (23.0)**	**60 (3.3)**	**584 (32.0)**	**1,827 (100.0)**

* Including high-risk HPV genotypes other than HPV 16/18 i.e.: HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous cell carcinoma, AC = Adenocarcinoma

Table 3: HPV genotype distribution by cervical pathology

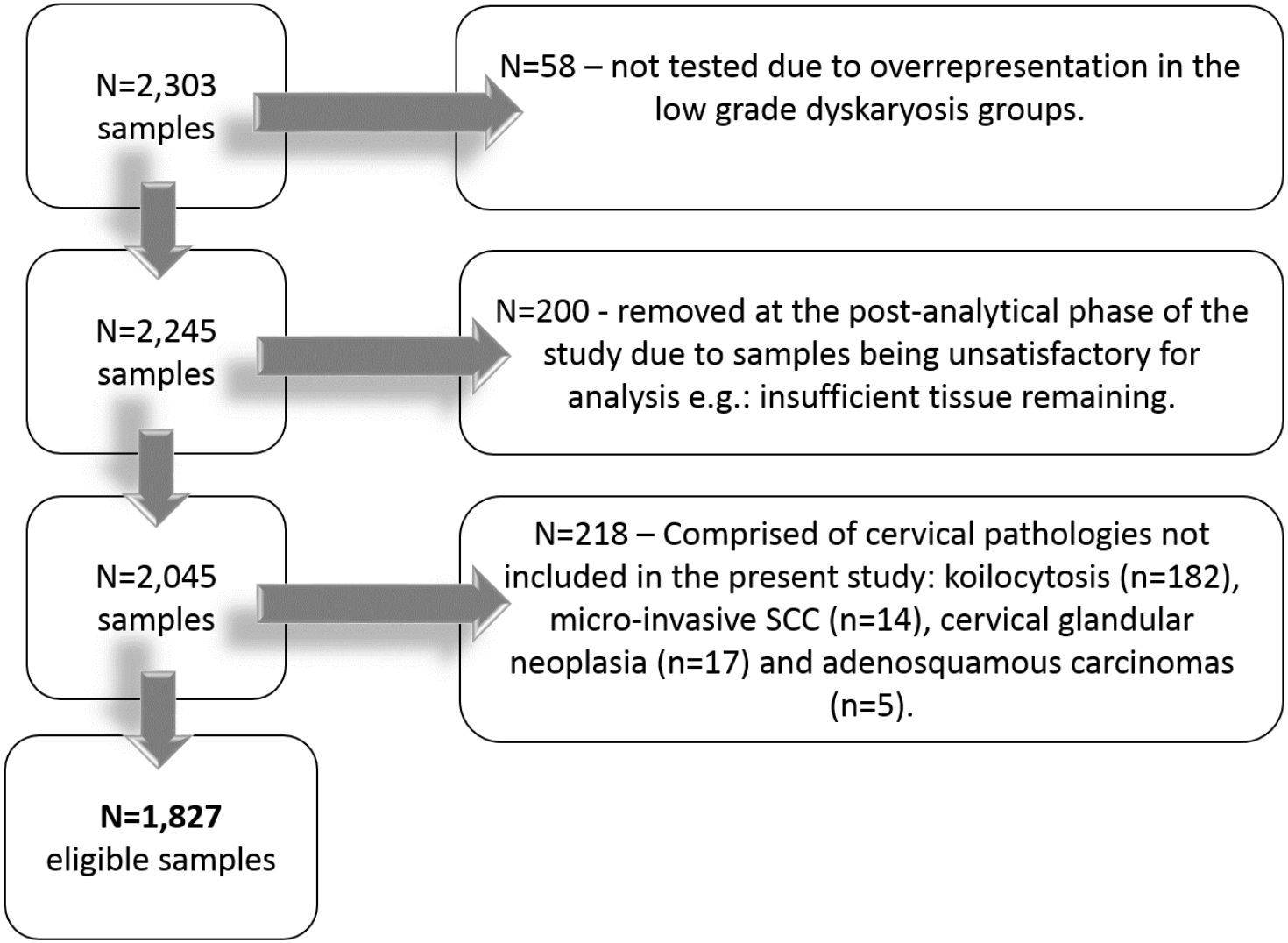
	Cervical histology					
HPV genotype	**AC**	**CIN I**	**CIN II**	**CIN III**	**SCC**	**Total**
HPV 16	3 (21.4)	87 (16.0)	136 (32.0)	407 (52.3)	51 (79.7)	684 (37.4)
HPV 31	0 (0.0)	35 (6.4)	29 (6.8)	84 (10.8)	2 (3.1)	150 (8.2)
HPV 52	0 (0.0)	22 (4.0)	33 (7.8)	65 (8.3)	5 (7.8)	125 (6.8)
HPV 18	6 (42.9)	29 (5.1)	17 (4.0)	39 (5.0)	3 (4.7)	93 (5.1)
HPV 33	0 (0.0)	18 (3.3)	15 (3.5)	58 (7.5)	0 (0.0)	91 (5.0)
HPV 51	1 (7.1)	17 (3.1)	25 (5.9)	28 (3.6)	0 (0.0)	71 (3.9)
HPV 45	0 (0.0)	14 (2.6)	10 (2.4)	31 (4.0)	3 (4.7)	58 (3.2)
HPV 39	0 (0.0)	20 (3.7)	12 (2.8)	14 (1.8)	1 (1.6)	47 (2.6)
HPV 58	0 (0.0)	11 (2.0)	13 (3.1)	17 (2.2)	0 (0.0)	41 (2.2)
HPV 66	0 (0.0)	22 (4.0)	6 (1.4)	10 (1.3)	0 (0.0)	38 (2.1)
HPV 35	0 (0.0)	9 (1.7)	12 (2.8)	16 (2.1)	0 (0.0)	37 (2.0)
HPV 59	0 (0.0)	14 (2.6)	8 (1.9)	8 (1.0)	0 (0.0)	30 (1.6)
HPV 56	0 (0.0)	18 (3.3)	3 (0.7)	5 (0.6)	0 (0.0)	26 (1.4)
HPV 6	0 (0.0)	7 (1.3)	9 (2.1)	9 (1.2)	0 (0.0)	25 (1.4)
HPV 73	0 (0.0)	9 (1.7)	6 (1.4)	8 (1.0)	0 (0.0)	23 (1.3)
HPV 53	0 (0.0)	9 (1.7)	5 (1.2)	5 (0.6)	0 (0.0)	19 (1.0)
HPV 70	0 (0.0)	4 (0.7)	4 (0.9)	9 (1.2)	0 (0.0)	17 (0.9)
HPV 42	0 (0.0)	9 (1.7)	2 (0.5)	3 (0.4)	0 (0.0)	14 (0.8)
HPV 61	0 (0.0)	6 (1.1)	1 (0.2)	7 (0.9)	0 (0.0)	14 (0.8)
HPV 54	1 (7.1)	2 (0.4)	3 (0.7)	5 (0.8)	0 (0.0)	12 (0.7)
HPV 11	0 (0.0)	5 (0.9)	2 (0.5)	4 (0.5)	0 (0.0)	11 (0.6)
HPV 68	0 (0.0)	4 (0.7)	5 (1.2)	2 (0.3)	0 (0.0)	11 (0.6)
HPV CP6108	0 (0.0)	4 (0.7)	0 (0.0)	6 (0.8)	0 (0.0)	10 (0.6)
HPV 82	0 (0.0)	1 (0.2)	0 (0.0)	8 (1.0)	0 (0.0)	9 (0.5)
HPV 62	0 (0.0)	2 (0.4)	1 (0.2)	4 (0.5)	0 (0.0)	7 (0.4)
HPV 84	0 (0.0)	3 (0.6)	2 (0.5)	2 (0.3)	0 (0.0)	7 (0.4)
HPV 81	0 (0.0)	6 (1.1)	0 (0.0)	1 (0.1)	0 (0.0)	7 (0.4)
HPV 69	0 (0.0)	2 (0.4)	1 (0.2)	2 (0.3)	0 (0.0)	5 (0.3)
HPV 55	0 (0.0)	3 (0.6)	1 (0.2)	0 (0.0)	0 (0.0)	4 (0.2)
HPV 83	0 (0.0)	2 (0.4)	1 (0.2)	1 (0.1)	0 (0.0)	4 (0.2)
HPV is39	0 (0.0)	0 (0.0)	2 (0.5)	1 (0.1)	0 (0.0)	3 (0.2)
HPV 67	0 (0.0)	3 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)
HPV 40	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)	0 (0.0)	2 (0.11)
HPV 26	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)	0 (0.0)	2 (0.1)
HPV 64	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
HPV 72	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
HPV 71	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TOTAL	11 (78.5)	400 (73.6)	364 (85.6)	861 (110.8)	65 (101.6)	1701 (93.3)*

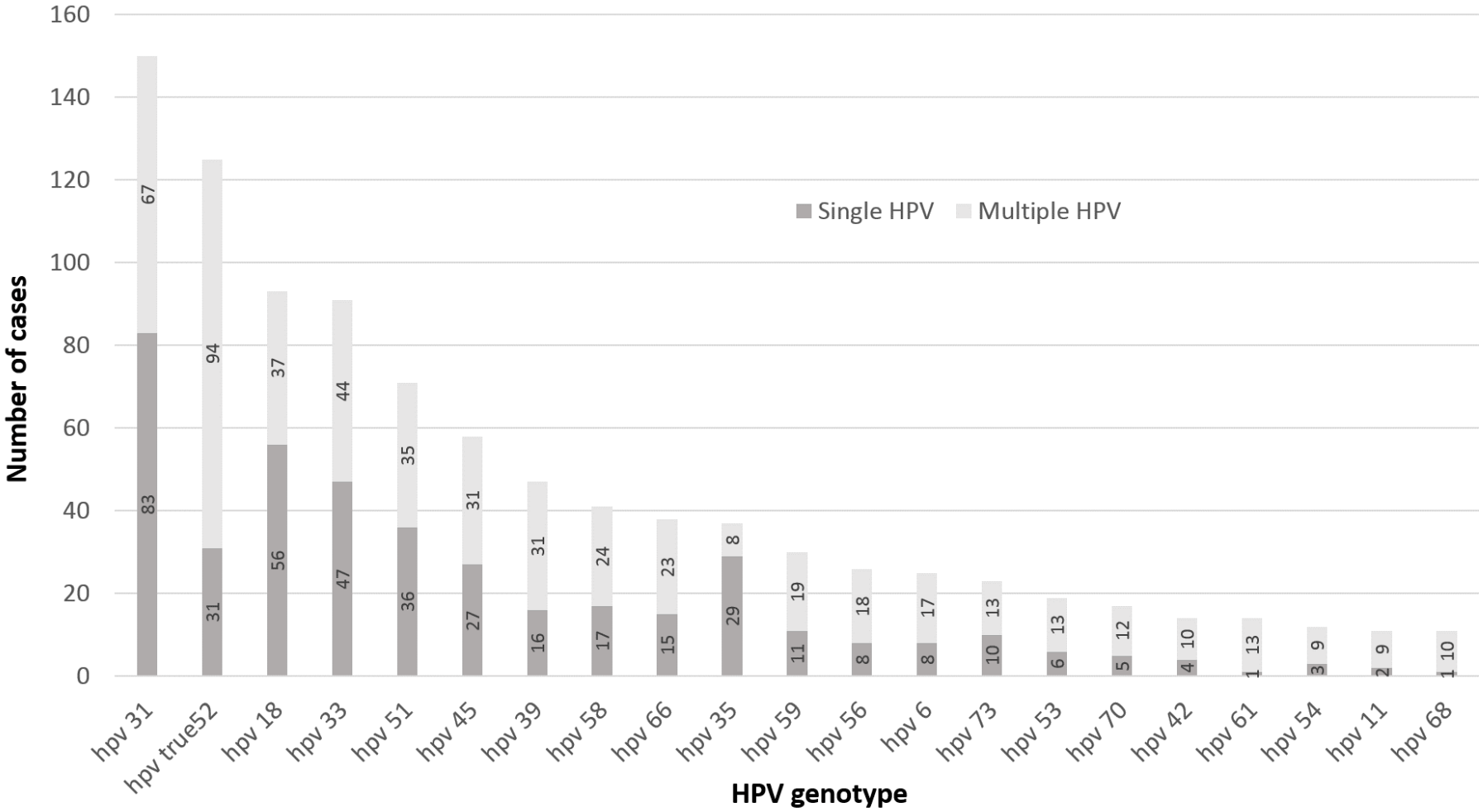
AC = adenocarcinoma, CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous Cell Carcinoma
* This figure does not add up to 1,243 (i.e.: the number HPV positive) as several patients may have had multiple HPV genotypes on testing

Table 4: Pathological distribution of cohort (numbers of cases) by five-year age group

	Mean age years (± SD; range)	**AGE** (years)										n (%)
PATHOLOGY n (%)		**<25**	**25-29**	**30-34**	**35-39**	**40-44**	**45-49**	**50-54**	**55-59**	**60-64**	**65+**	**TOTAL**
CIN I	32 (10; 16-69)	144 (26.4)	136 (25.0)	82 (15.1)	55 (10.1)	51 (9.4)	42 (7.7)	19 (3.5)	11 (2.0)	3 (0.6)	2 (0.4)	545 (100.0)
CIN II	32 (9; 18-80)	83 (19.5)	120 (28.2)	92 (21.7)	51 (12.0)	40 (9.4)	17 (4.0)	11 (2.6)	6 (1.4)	1 (0.2)	4 (0.9)	425 (100.0)
CIN III	31 (8; 18-64)	146 (18.7)	268 (34.4)	169 (21.7)	94 (12.1)	53 (6.8)	27 (3.5)	12 (1.5)	7 (0.9)	3 (0.4)	0 (0.0)	779 (100.0)
SSC	43 (14; 23-93)	1 (1.6)	3 (4.7)	12 (18.8)	19 (29.7)	7 (10.9)	5 (7.8)	7 (10.9)	3 (4.7)	1 (1.6)	6 (9.4)	64 (100.0)
AC	42 (16; 23-73)	1 (7.1)	2 (14.3)	3 (21.4)	3 (21.4)	0 (0.0)	1 (7.1)	0 (0.0)	1 (7.1)	1 (7.1)	2 (14.3)	14 (100.0)
TOTAL n (%)		375 (20.5)	529 (28.9)	358 (19.6)	222 (12.2)	151 (8.3)	92 (5.0)	49 (2.7)	28 (1.5)	9 (0.5)	14 (0.8)	1,827 (100.0)

CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous Cell Carcinoma, AC= Adenocarcinoma
±SD = Standard deviation
N.B.: Please note that certain pathological groups were overrepresented in this study, so the proportions above may not be a true reflection of the distribution of cervical pathologies within each age category in Northern Ireland.


N=2,303 samples
N=58 – not tested due to overrepresentation in the low grade dyskaryosis groups.
N=2,245 samples
N=200 - removed at the post-analytical phase of the study due to samples being unsatisfactory for analysis e.g.: insufficient tissue remaining.
N=2,045 samples
N=218 – Comprised of cervical pathologies not included in the present study: koilocytosis (n=182), micro-invasive SCC (n=14), cervical glandular neoplasia (n=17) and adenosquamous carcinomas (n=5).
N=1,827 eligible samples



HPV genotypes omitted above (no. of single/multiple infections): HPV 16 (480/204), HPV CP6108 (1/9), HPV 82 (0,9), HPV 62 (0/7), HPV 84 (1/6), HPV 81 (3/4), HPV 69 (1/4), HPV 55 (0/4), HPV 83 (2/2), HPV IS39 (1/2), HPV 67 (2/1), HPV 40 (0/2), HPV 26 (1/1), HPV 64 (0/1), HPV 72 (0/0), HPV 71 (0/0).